Figures 3A-3B. Predicted amino acid sequence of C-*Delta*-1 (SEQ ID NO:2), aligned with that of X-*Delta*-1 (*Xenopus Delta*; SEQ ID NO:5) and *Drosophila Delta* (SEQ ID NO:6) and, indicating the conserved domain structures: EGF repeats, DSL domain, and transmembrane domain (TM). Conserved amino acids are boxed, and ● denote aligned and non-aligned N-terminal cysteine residues, respectively. Although the intracellular domains of C-*Delta*-1 and X-*Delta*-1 closely resemble each other, they show no significant homology to the corresponding part of *Drosophila Delta*.

On page 9, replace the paragraph beginning "Figure 7" with the following:

Figures 7A-7B. The DNA sequence of mouse *Delta* (M-*Delta*-1) (SEQ ID NO:11).

On page 9, replace the paragraph beginning "Figure 9" with the following:

Figures 9A-9B. An alignment of the predicted amino acid sequence of mouse M-*Delta*-1 (SEQ ID NO:12) with the chick C-*Delta*-1 (SEQ ID NO:2) which shows their extensive amino acid sequence identity. Identical amino acids are boxed. The consensus sequence between the two genes is at the bottom (SEQ ID NO:13).

On page 10, replace the paragraph beginning "Figure 10" with the following:

Figures 10A-10B. The DNA sequence of a PCR amplified fragment of human *Delta* (H-*Delta*-1) (SEQ ID NO:14) and the predicted amino acid sequences using the three available open reading frames, 2nd line (SEQ ID NOS:15-17), 3rd line (SEQ ID NO:18), 4th line (SEQ ID NOS:19-22).

On page 10, replace the paragraph beginning "Figure 11" with the following:

Figure 11. An alignment of human H-*Delta*-1 (top line) and chick C-*Delta*-1 (bottom line). The predicted amino acid sequence of human *Delta* (SEQ ID NO:23) is shown in the top line. The sequence of human *Delta* was determined by "eye", in which the sequence of the appropriate reading frame was determined by maximizing homology with C-*Delta*-1. No single reading frame shown in Figures 10A-10B gave the correct sequence due to errors in the DNA sequence of Figures 10A-10B that caused reading frameshifts.

On page 10, replace the paragraph beginning "Figure 12A-12B" with the following:

Figures 12A1-12A3 - 12B1-12B6. Figures 12A1-12A3 present the contig DNA sequence of human *Delta* (H-*Delta*-1) (SEQ ID NO:26) from clone HDl 18. Figures 12B1-12B6 present the nucleotide sequence shown in Figures 12A1-12A3 (top line, SEQ ID NO:26) and the deduced amino acid sequences using the three possible open reading frames, second line (SEQ ID NOS:27-42), third line (SEQ ID NOS:43-47), fourth line (SEQ ID NOS:48-64). The amino acid sequence with the greatest homology to the mouse *Delta*-1 amino acid sequence is boxed. This boxed amino acid sequence is the predicted amino acid sequence of human *Delta*; where the reading frame shifts indicates where a sequencing error is present in the sequence. No single reading frame shown in Figures 12A1-12A3 gave an uninterrupted amino acid sequence due to errors in the DNA sequence that caused shifts in the reading frame. X indicates an undetermined amino acid; N indicates an undetermined nucleotide.

On page 10, replace the paragraph beginning "Figure 13" with the following:

Figures 13A-13G. An alignment of mouse M-*Delta*-1 DNA sequence (top line, SEQ ID NO:4) and human H-*Delta*-1 DNA sequence (second line, SEQ ID NO:26) and their consensus sequence (third line, SEQ ID NO:24).

On page 11, replace the paragraph beginning "Figure 14" with the following:

Figures 14A-14B. The composite human *Delta* (H-*Delta*-1) amino acid sequence (SEQ ID NOS:65-80, respectively) is presented, representing the boxed amino sequence from Figures 12B1-12B6. ">" indicates that the sequence continues on the line below. "*" indicates a break in the sequence.

On page 13, replace the paragraph beginning "The invention relates" with the following:

The invention relates to the nucleotide sequences of vertebrate *Delta* nucleic acids. In specific embodiments, human *Delta* nucleic acids comprise the cDNA sequences shown in Figures 10A-10B (SEQ ID NO:14) or in Figures 12A1-12A3 (SEQ ID NO:26), or the coding regions thereof, or nucleic acids encoding a vertebrate *Delta* protein (e.g., having the sequence of SEQ ID NO:1, 3, 11, 14 or 26). The invention provides nucleic acids

consisting of at least 8 nucleotides (i.e., a hybridizable portion) of a vertebrate *Delta* sequence; in other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a *Delta* sequence, or a full-length *Delta* coding sequence. The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences or their complements. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a vertebrate *Delta* gene. In a specific embodiment, a nucleic acid which is hybridizable to a vertebrate (e.g., mammalian) *Delta* nucleic acid (e.g., having sequence SEQ ID NO:14 or SEQ ID NO:26, or an at least 10, 25, 50, 100, or 200 nucleotide portion thereof), or to a nucleic acid encoding a *Delta* derivative, under conditions of low stringency is provided. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X $10^6$ cpm $^{32}$P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art (e.g., as employed for cross-species hybridizations).

On page 15, replace the paragraph beginning "Fragments of vertebrate" with the following:

Fragments of vertebrate *Delta* nucleic acids comprising regions of homology to other toporythmic proteins are also provided. The DSL regions (regions of homology with *Drosophila* Serrate and *Delta*) of *Delta* proteins of other species are also provided. Nucleic acids encoding conserved regions between *Delta* and Serrate, such as those shown in Figures 3A-3B and 8 are also provided.

On page 26, replace the paragraph beginning "In a specific embodiment" with the following:

B12

In a specific embodiment of the present invention, such *Delta* proteins, whether produced by recombinant DNA techniques or by chemical synthetic methods, include but are not limited to those containing, as a primary amino acid sequence, all or part of the amino acid sequences substantially as depicted in Figures 2, 8, 11 or 14A-14B (SEQ ID NOS:2, 12, 23 and 65-80), as well as fragments and other derivatives, and analogs thereof.

On page 29, replace the paragraph beginning "Various procedures" with the following:

B13

Various procedures known in the art may be used for the production of polyclonal antibodies to a *Delta* protein or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the *Delta* protein encoded by a sequence depicted in Figures 1A1-1A3, 1B1-1B2, 7A-7B or 11, or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native *Delta* protein, or a synthetic version, or derivative (e.g., fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

On page 36, replace the paragraph beginning "In a specific embodiment" with the following:

B14

In a specific embodiment, the invention relates to vertebrate *Delta* derivatives and analogs, in particular *Delta* fragments and derivatives of such fragments, that comprise, or alternatively consist of, one or more domains of the *Delta* protein, including but not limited to the extracellular domain, signal sequence, region amino-terminal to the DSL domain, DSL domain, ELR domain, transmembrane domain, intracellular domain, and one or more of the EGF-like repeats (ELR) of the *Delta* protein (e.g., ELRs 1-9), or any combination of the foregoing. In particular examples relating to the

chick and mouse *Delta* proteins, such domains are identified in Examples Section 6 and 7, respectively, and in Figures 3A-3B and 9A-9B. Thus, by way of example is provided, a molecule comprising an extracellular domain (approximately amino acids 1-545), signal sequence (approximately amino acids 1-17), region amino-terminal to the DSL domain (approximately amino acids 1-178), the DSL domain (approximately amino acids 179-223), EGF1 (approximately amino acids 229-260), EGF2 (approximately amino acids 261-292), EGF3 (approximately amino acids 293-332), EGF4 (approximately amino acids 333-370), EGF5 (approximately amino acids 371-409), EGF6 (approximately amino acids 410-447), EGF7 (approximately amino acids 448-485), EGF8 (approximately amino acids 486-523), transmembrane domain, and intracellular (cytoplasmic) domain (approximately amino acids 555-728) of a vertebrate *Delta*.

On page 68, replace the paragraph beginning "We identified" with the following:

We identified a chick *Delta* homologue, C-*Delta*-1, using the polymerase chain reaction (PCR) and degenerate oligonucleotide primers (Figures 1A1-1A3, 1B1-1B2 and 2, SEQ ID NOS:1, 2, 3 and 4). C-*Delta*-1 was cloned by PCR using the degenerate oligonucleotide primers TTCGGITT(C/T)ACITGGCCIGGIAC (SEQ ID NO:81) and TCIATGCAIGTICCICC(A/G)TT (SEQ ID NO:82) which correspond to the fly *Delta* protein sequences FGFTWPGT (SEQ ID NO:83) and NGGTCID (SEQ ID NO:84), respectively (Vässin et al., 1987, EMBO J. 6:3431-3440; Kopczynski et al., 1988, Genes Dev. 2:1723-1735). The initial reaction used 50ng of first-strand oligo-d(T)-primed cDNA from stage 4-6 embryos, 1 g of each primer, 0.2mM dNTPs, 2U of Taq polymerase, in 50 l of the supplied buffer (Perkin-Elmer). 40 cycles of amplification were performed at 94°C/30sec; 50°C/2min; 72°C/2min. Amplified DNA fragments were separated on an agarose gel, cloned in Bluescript pKS- (Stratagene) and sequenced. Two *Delta* homologs were identified, one of which (C-*Delta*-1) is expressed in the nervous system. Of the homolog that is expressed in the nervous system, two variants were identified that differ at the carboxy-terminal end of the encoded protein due to an alternative splicing event at the 3' end of the C-*Delta*-1 gene. One encoded protein has 12 extra amino acids at the carboxy-terminal end, relative to the other encoded protein. The sequence of the shorter encoded variant is set forth in SEQ ID NO:2. The longer variant encoded by SEQ ID NO:3 and identified by the amino acid sequence of SEQ ID NO:4, consists of the amino acid

sequence of SEQ ID NO:2 plus twelve additional amino acids at the 3' end (SIPPGSRTSLGV) (SEQ ID NO:85). The longer variant was used in the experiments described below. When tested for biological activity by injection of RNA into *Xenopus* oocytes, each of the variants had the same biological activity.

On page 68, replace the paragraph beginning "DNA fragments" with the following:

DNA fragments corresponding to C-*Delta*-1 were used to screen a stage 17 spinal cord cDNA library and several full-length clones were obtained and sequenced. We amplified DNA fragments from chick C-*Notch*-1 gene by similar methods (data not shown); partial sequence data and pattern of expression indicate close similarity to the rodent Notch-1 gene (Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Lardelli & Lendahl, 1993, Exp. Cell Res. 204:364-372). Sequences were analyzed using the Wisconsin GCG set of programs. The GenBank Accession number for the Chick *Delta*-1 mRNA is U26590. The DNA sequence of C-*Delta*-1 corresponds to a protein of 722 amino acids, structurally homologous to *Drosophila Delta* (Figures 3A-3B, 4) and clearly distinct from vertebrate homologs of the *Delta*-related Serrate protein, which we have also cloned (data not shown). C-*Delta*-1 contains a putative transmembrane domain, a signal sequence and 8 EGF-like repeats in its extracellular region (one repeat less than *Drosophila Delta*). The amino-terminal domain of C-*Delta*-1 is closely related to a similar domain in the fly *Delta* protein, described as necessary and sufficient for in vitro binding to Notch (Muskavitch, 1994, Dev. Biol. 166:415-430). This conserved region includes the so-called DSL motif (Fig. 4) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154), shared by all known members of the family of presumed ligands of Notch-like proteins (*Delta* and Serrate in *Drosophila*; Lag-2 and Apx-1 in *Caenorhabditis elegans*) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154; Fleming et al., 1990, Genes Dev. 4:2188-2201; Thomas et al., 1991, Development 111:749-761; Mello et al., 1994, Cell 77:95-106). A second cysteine-rich N-terminal region is conserved between the fly and chick proteins, but absent from the related *C. elegans* proteins (Fig. 4). The *Xenopus Delta*-1 homologue, X-*Delta*-1 which encodes a protein that is 81% identical to C-*Delta*-1 and shows all the above structural motifs (Figures 3A-3B), has also been cloned. The structural conservation between the chick and fly *Delta* proteins, including domains

identified as critical for Notch binding (Muskavitch, 1994, Dev. Biol. 166:415-430), suggests that C-*Delta*-1 functions as a ligand for a chick Notch protein, and that a *Delta*/Notch-mediated mechanism of lateral inhibition might operate in the chick.

On page 75, replace the paragraph beginning "Figure 7" with the following:

Figures 7A-7B (SEQ ID NO:11) show the nucleotide sequence of the isolated clone containing M-*Delta*-1 DNA.

On page 75, replace the paragraph beginning "Figure 9 shows" with the following:

Figures 9A-9B show an amino acid alignment of the predicted amino acid sequences for M-*Delta*-1 and C-*Delta*-1. Identical amino acids are boxed showing the extensive sequence homology. The consensus sequence is shown below (SEQ ID NO:13).

On page 76, replace the paragraph beginning "A human genomic" with the following:

A human genomic library with inserts ranging in size from 100-150 kb was probed with an EcoRI fragment of the mouse *Delta*-1 (M-*Delta*-1) gene. From the library a genomic human PAC clone was isolated which hybridized to the EcoRI fragment. Next, two degenerate oligonucleotides were used to amplify by PCR a fragment of the genomic human PAC clone. The degenerate oligos were:

5' ACIATGAA(C/T)AA(C/T)CTIGCIAA(C/T)TG (SEQ ID NO:89) [encoding TMNNLANC (SEQ ID NO:90)] and

3' AC(A/G)TAIACIGA(C/T)TG(A/G)TA(C/T)TTIGT (SEQ ID NO:91) [encoding TKYQSVYV (SEQ ID NO:92)] or

3' GC(A/G/T)ATIAC(A/G)CA(C/T)TC(A/G)TC(C/T)TT(C/T)TC (SEQ ID NO:93) [encoding EKDECVIA (SEQ ID NO:25)].

On the basis of the cDNA sequences for chicken and mouse *Delta*-1, it was expected that fragments of approximately 354 and 387 base pairs would be isolated, using the 5' and the two different 3' oligos, respectively. In fact, however, two single isolates of 525 base pairs and another that was 30 base pairs smaller, as expected, were obtained. The larger isolate was sequenced by dideoxy sequencing. The nucleotide sequence is shown in Figures 10A-10B (SEQ ID NO:14). Also shown in Figures 10A-10B are the predicted amino acid

sequences of the amplified DNA fragment (SEQ ID NOS:15-22) for the three different readings frames. Due to sequencing errors, the full uninterrupted sequence between both primers was not identified. As a consequence, one cannot predict the amino acid sequence directly from the DNA sequence obtained. However, Figure 11 shows the amino acid sequence homology between human *Delta*-1 (top line) (SEQ ID NO:23) and chick *Delta*-1 (bottom line) as determined by eye. Because of the sequencing errors, the homology was obtained by switching amongst the three different reading frames to identify the homologous regions.

On page 77, replace the paragraph beginning "Since the sequence" with the following:

Since the sequence thus obtained did not contain the 5' end of HDl, HDl24 was used as a probe for subsequent hybridizations in a T cell library and in another fetal brain library (Lambda-Zap, Stratagene). A screen of the T cell library resulted in no positives. However, screening the Lambda-Zap library resulted in two positive clones, HDl13 and HDl18. These clones were inserted into a Bluescript KS- vector using *Eco*RI as described above. The inserts were digested with a panel of restriction enzymes for comparison with HDl3 and HDl24, and the 5' and 3' ends were sequenced using T3 and T7 primers. HDl13 was determined to be only a small piece of cDNA that when sequenced showed no homology to any known *Delta*. However, HDl18 was 3 kb in length, and included the entire sequence of HDl24 with additional 5' sequences. A set of clones were isolated using nested deletions from HDl18; these clones were then subjected to dideoxy sequencing using an automated sequencer. Figures 12A1-12A3 present the partial nucleotide contig sequence (SEQ ID NO:26) of human *Delta* obtained from clone HDl18. Due to sequencing errors, the full uninterrupted nucleotide sequence of human *Delta* was not determined. Figures 12B1-12B6 show the partial nucleotide contig sequence (SEQ ID NO:26) of human *Delta* (top line), with the predicted amino acid sequence in three different reading frames presented below, the second line being reading frame 1 (SEQ ID NOS:27-42), the third line being reading frame 2 (SEQ ID NOS:43-47), and the fourth line being reading frame 3 (SEQ ID NOS:48-64).

On page 78, replace the paragraph beginning "Sequence homology" with the following:

B21

Sequence homology was determined by eye using the mouse *Delta*-1 amino acid sequence. The sequences with the greatest degree of homology to the mouse amino acid sequence are boxed in Figures 12B1-12B6, and represent the predicted amino acid sequence of human *Delta*-1. The composite resulting amino acid sequence is shown in Figures 14A-14B. (In Figures 14A-14B, the various uninterrupted portions of the human *Delta* sequence are assigned respectively, SEQ ID NOS:65-80.) Note that due to sequencing errors, the reading frame with the greatest homology is not the same throughout the sequence and shifts at positions where there are errors in the sequence.

On page 78, replace the paragraph beginning "Figure 13 presents" with the following:

B22

Figures 13A-13G present the nucleotide sequence of mouse *Delta*-1 (top line, SEQ ID NO:4) and the contig nucleotide sequence of human *Delta*-1 as depicted in Figures 12A1-12A3 and 12B1-12B6 (second line, SEQ ID NO:26) and the nucleotide consensus sequence between mouse and human *Delta* (third line, SEQ ID NO:24).

On page 79, replace the paragraph beginning "Using probes containing" with the following:

B23

Using probes containing the human *Delta* 5' nucleotide sequences presented in Figures 12A1-12A3, cDNA libraries are probed to isolate the 5' end of the human *Delta* gene. Primary positive clones are obtained and then confirmed as secondary positives. The secondary positives are purified and grown further. The DNA is then isolated and subcloned for sequencing.